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Plenary

P1

Lactose permease: A beautiful chemiosmotic machine

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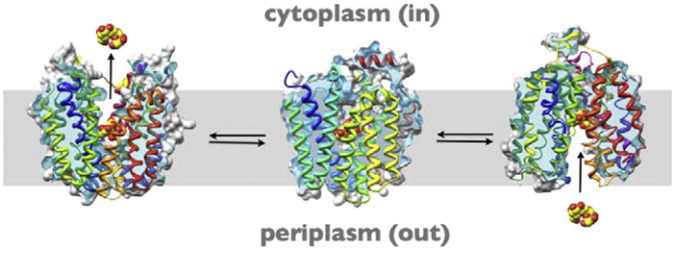
The lactose permease (LacY), a paradigm for membrane transport proteins and a member of the Major Facilitator Superfamily, catalyzes the coupled, stoichiometric translocation of a galactoside and an H^+ across the cytoplasmic membrane of *Escherichia coli*. Thus, sugar accumulation against a concentration gradient is driven by the free energy released from the downhill movement of H^+ with the electrochemical H^+ gradient ($\Delta\tilde{\mu}_H+$; interior negative and/or alkaline). Moreover, since transport is obligatorily coupled, downhill sugar translocation drives uphill H^+ translocation with the generation of $\Delta\tilde{\mu}_H+$, the polarity of which depends on the direction of the sugar concentration gradient. X-ray crystal structures reveal an inward-facing conformation and confirm many conclusions from biochemical and biophysical experiments. LacY contains N- and C-terminal domains, each with 6 largely irregular transmembrane helices positioned pseudo-symmetrically and surrounding a deep water-filled cavity open to the cytoplasm only. Sugar- and H^+-binding sites are located primarily in the N- and C-terminal helix bundles, respectively, at the apex of the cavity in the approximate middle of the molecule, and the periplasmic side is tightly sealed. Every residue in LacY has been mutagenized, and those involved in sugar and H^+ binding have been identified. Surprisingly, those involved in H^+ binding and translocation are aligned parallel to the membrane at the same level as the sugar-binding site. Both sites are exposed reciprocally to water-filled cavities in the inward- or outward-facing conformation, thereby allowing sugar and H^+ release from either side of LacY via an alternating access mechanism. These features likely explain how LacY catalyzes lactose/H^+ symport in both directions across the membrane utilizing the same residues. Site-directed alkylation, single molecule FRET, double electron–electron resonance, thiol cross-linking and Trp quenching/unquenching studies provide converging evidence for an alternating access mechanism. The primary driving force for alternating access is sugar binding and dissociation, and $\Delta\tilde{\mu}_H+$ changes the rate-limiting step. Evidence for an occluded intermediate will also be discussed.



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P2

The ATP synthase: The understood, the uncertain and the unknown

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The F-ATPases, or F_1F_o-ATPases, are multisubunit enzyme complexes found in energy transducing membranes in eubacteria, mitochondria and chloroplasts. Their role is to synthesize ATP from ADP and phosphate under aerobic conditions using the proton-motive force generated by respiration or photosynthesis as a source of energy. The ATP hydrolase activities of the enzymes from mitochondria, chloroplasts and some eubacteria are inhibited, and they can only synthesize ATP. However, other eubacterial enzymes hydrolyze ATP, made by glycolysis under anaerobic conditions, to generate the proton motive force required for essential cellular functions, such as chemotaxis and transmembrane transport processes. The lecture will describe the common features and differences between the F-ATPases from these various sources. It has been assumed widely that information from one F-ATPase would apply to all or many other F-ATPases, but it is increasingly evident that while there are common principles in the operation of F-ATPases from diverse sources, there are also significant differences. This is most evident in the variety of the symmetries of the membrane bound c-rings in the rotors of the enzyme from various species, which has profound consequences for the bioenergetic cost of making ATP.

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P3

Structure, function and regulation of ion pumps

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The P-type ATPases undergo large conformational changes via formation and breakdown of a phosphoenzyme intermediate coupled to a functional cycle illustrating the alternating access model for active transport. Worth noting, these large-scale movements take place in a close interaction with the membrane [4]. Recently we have obtained further insight of the conformational changes from a new structure representing the E1 state of the sarcoplasmic reticulum Ca^{2+}-ATPase (Winther, Bublitz et al., unpublished).

The Na^+,K^+-ATPase imposes a number of fundamental questions on how P-type ATPases work, not least due to its complex mechanism transporting three sodium ions out of the cell and two potassium ions in per ATPase cycle. From the crystal structure of the pig kidney Na^+,K^+-ATPase enzyme in the potassium-bound form and from mutational studies we identified a key role of the alpha subunit C-terminus on sodium binding [2]. Electrophysiologial studies pointed to a specific role in a C-terminal proton pathway leading to the ion binding sites with significant implications to neurological diseases [3]. Recently we determined the first crystal structure of a sodium-bound, E1P-like form of Na^+,K^+-ATPase (unpublished) providing new insight of the formation and control of three sodium sites. Furthermore, a crystal structure of the phosphorylated E2P form of the Na^+,K^+-ATPase in complex with the cardiotonic steroid ouabain shows conformational changes that not only explain the mechanism of inhibition, but also hint at the possible role and specific binding of the cardiotonic steroids in signaling and how potassium and steroids antagonize each other [5] and unpublished.

The P1B-ATPases are expected to use similar modes of conformational changes, although with several unique features on the specific binding and transport of transition metals as revealed from our studies of the copper exit pathway of the Cu^+-ATPase [1], and unpublished.

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P4

Redox tuning in bioenergetics: Compromising efficiency to survive life in O_2

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Energy-converting redox enzymes perform productive reactions efficiently despite the involvement of high energy intermediates in their catalytic cycles. This is achieved by kinetic control: with forward reactions being faster than competing, energy-wasteful reactions. This requires appropriate cofactor spacing, driving forces and reorganizational energies [1]. These features evolved in ancestral enzymes in a low O_2 environment [2]. When O_2 appeared, energy-converting enzymes had to deal with its troublesome chemistry. Various protective mechanisms duly evolved that are not directly related to the enzymes' principal redox roles. These protective mechanisms involve fine-tuning of reduction potentials, switching of pathways and the use of short circuits, back-reactions and side-paths, all of which compromise efficiency [2]. This energy is worth it as it minimises damage from reactive derivatives of O_2 and thus gives the organism a better chance of survival. Many properties of photosynthetic reaction centres, some of them previously unexplained, can be rationalised in this context, e.g. the heterodimeric nature of Photosystem 1 (PS1) and the properties of the anaerobic PS1 variant in N_2 fixing species [2]. The redox tuning is aimed at avoiding the two key physicochemical values associated with life in oxygen: 1 eV (the energy that converts $^3O_2$ to $^1O_2$) and −160 mV (the potential that reduces O_2 to $O_{2-\cdot}$). This "sacrifice-of-efficiency-for-protection" concept should be generally applicable to bioenergetic enzymes in aerobic environments. It should also be taken into account for engineering improved efficiency into electron transfer proteins and for the design of artificial redox enzymes. It seems likely that engineering changes into photosynthetic reaction centres (and other electron transfer proteins) aimed at optimising rates, yields or overall chemical outcomes through changes in redox and/or colour will pay a penalty at the level of increased oxidative damage.

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P5

High resolution structure of photosystem II and the mechanism of water-splitting

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Photosystem II is a multi-subunit membrane protein complex containing 19–20 subunits with a total molecular mass of 350 kDa, and forms a dimer in cyanobacterial cells. PSII catalyzes light-induced water splitting, leading to the evolution of molecular oxygen. The catalytic center of water-oxidation is a Mn_4CaO_5-cluster. We have resolved the crystal structure of photosystem II (PSII) from *Thermosynechococcus vulcanus* at 1.9 Å resolution [1]. In this talk, I will summarize the high resolution structure of PSII with the emphasis on the structure of the oxygen-evolving complex (OEC), which is organized in a distorted chair form with a cubane-like structure composed of Mn_3CaO_4 linked via μ-oxo bridges to a fourth Mn (Mn4) outside of the cubane. The distortion in the cubane